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FINAL REPORT

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OBJECTIVE: To characterize the molecular mechanisms controlling substratum recognition, adhesion and metamorphosis of the larvae of model macrofouling organisms on surfaces immersed in the ocean.

ACCOMPLISHMENTS : We advance both our *in vitro* dissection of the molecular mechanisms controlling larval attachment and metamorphosis of the mollusc, *Haliotis rufescens*, (D. Morse, 1990, 1991, 1992; A. Morse, 1991; Wodicka and Morse, 1991; Baxter and Morse, 1992), and our characterization of the molecular adhesive and the mechanisms controlling fouling by the cementing polychaete, *Phragmatopoma californica* (Jensen and Morse, 1990; Jensen et al., 1990; Waite et al, 1992; Jensen, 1992; Ilan et al., 1992). **Our results show that the chemosensory mechanisms and the internal signal transducers mediating the induction of larval attachment and metamorphosis in both *Phragmatopoma* and *Haliotis* larvae are highly related at the molecular level, thus providing evidence from 2 phyla that the molecular mechanisms regulating the initial steps in macrofouler attachment are generic.**

Cilia purified from the epithelium of *Haliotis* larvae were found to contain the chemosensory receptors and signal transducers controlling substratum-specific attachment and metamorphosis in response to two classes of chemical signals from the environment: a peptide signal associated with the recruiting surfaces, and an amino acid present in variable concentrations in seawater. The (lysine) amino acid receptor and its signal transducing cascade of receptor-regulated G protein, phospholipase C and protein kinase C were resolved and analyzed *in vitro* (Morse, 1990, 1991, 1992; Baxter and

Morse, 1992). Reciprocal control of the chemosensory receptor by its G protein suggests that the receptor may be a transmembrane protein of the rhodopsin superfamily. The purified chemosensory cilia were found to contain mRNA encoding elements of the chemosensory pathway. Synthesis, PCR-amplification, cloning and sequence analysis of the corresponding cDNAs led to identification of two G protein mRNAs in the chemosensory cilia (Wodicka and Morse, 1991). One of these is closely homologous to the Gq family recently found in mammalian brain, and also shown to regulate phospholipase C. This pathway in the larvae amplifies the settlement response to the surface-associated inducers. Our nucleic acid studies in the larvae were facilitated by our development of a new technique for purification of larval mRNA and production of full-length larval cDNAs (Groppe and Morse, 1992a, 1992b). These studies also demonstrated that larval metamorphosis induces the expression of potent serine proteases; cDNA sequence analyses and Northern hybridizations show that these enzymes are members of a unique ancestral chymotrypsin family, highly and specifically expressed in the newly differentiated cells of the distal intestine.

We have shown that the adhesive protein secreted as a tube cement by *Phragmatopoma* is the chemical inducer of gregarious larval settlement of this macrofouler. In collaboration with Professor H. Waite (U. DE) we have accomplished the biochemical purification and sequence analyses of the principal DOPA-containing peptides of this adhesive protein precursor molecule (Waite, Jensen and Morse, 1992; Jensen,1992). These adhesive proteins are more closely related, at the sequence level, to the DOPA proteins of marine parasite eggshells than to the adhesive proteins of the marine mussel. We have shown that these proteins, and a chemical analog of their DOPA crosslinks, are potent inducers of substratum specific larval settlement both in the laboratory and in the natural ocean environment (Jensen and Morse, 1990). We also have shown that fatty acids are not present in the natural inducer; although these compounds can be obtained as contaminants of the inducer artifactually, they induce larval settlement non-specifically (Jensen et al., 1990). We recently have discovered a calcium-dependent control of metamorphosis in *Phragmatopoma*, possibly involving protein kinase C (Ilan et al., 1992). The calcium-dependent signal transducers mediating the induction of *Phragmatopoma* larval attachment and metamorphosis prove highly related to those controlling these processes in the *Haliotis* larvae, thus providing evidence from 2 phyla that the molecular mechanisms regulating the initial steps in macrofouler attachment are generic.

SIGNIFICANCE: Our results demonstrate that purified larval cilia provide a model system uniquely suited for *in vitro* resolution of the chemosensory receptors and signal transducers controlling metamorphosis in planktonic marine invertebrate larvae. Our discovery that functional mRNA can be

purified from these cilia in quantities sufficient to establish a cDNA library extends the tractability of the *Haliotis* larval system to analyses of the chemosensory elements at the cDNA and protein sequence level. This is the first discovery of functional mRNA in cilia from any system, and itself is likely to open new areas of research in many laboratories. Results of these studies are helping to elucidate the detailed molecular mechanisms by which chemosensory receptors and transducers regulate larval settlement behavior and metamorphosis. Results of the continuing cDNA investigations should provide insights into the basic mechanisms of action (and the evolution) of the chemoreceptors and their associated signal-transducers in the molluscan larvae. The mechanisms by which these control responsiveness to stimuli, physiological and behavioral processes, and the activation of gene expression and development, can be expected to be applicable to a wide variety of other sensory, neuronal, hormonal and developmental systems as well. These results may help us identify new targets and strategies for the prevention of larval settlement attachment and biofouling through non-polluting means. The structural characterization of the adhesive protein from *Phragmatopoma* may lead to the development of useful new composite biomaterials, including underwater and medically useful adhesives.

Our finding that the chemosensory mechanisms and transducers controlling larval attachment and metamorphosis are closely related, at the molecular level, for species from two phyla (including a major macrofouler) substantiates the earlier suggestions that the molecular components controlling the initial stages of macrofouler attachment are in fact generic, and thus best studied in the most tractable model systems available.

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